MYOCARDIAL SUBSTRATE UTILIZATION IN DOGS FOLLOWING ENDOTOXIN ADMINISTRATION

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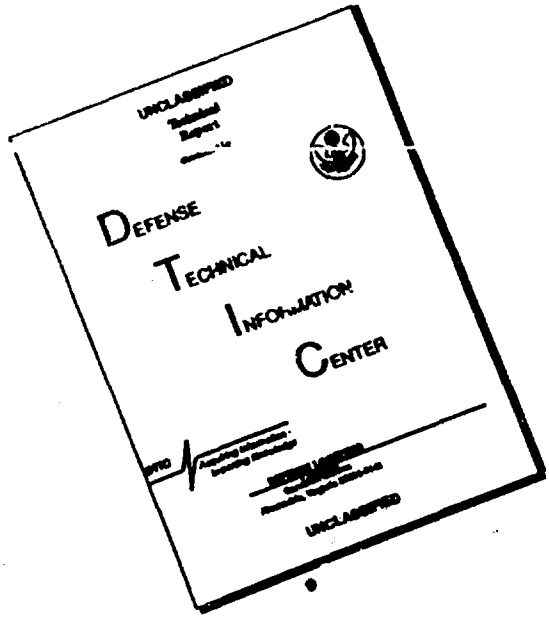

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13. ABSTRACT

Changes of myocardial substrate utilization have been studied in dogs under pentobarbital anesthesia following the administration of E. coli endotoxin. Myocardial substrate utilization under control conditions, at two hours, and again at three to four hours following endotoxin administration were compared. At both periods following endotoxin mean arterial blood pressure, heart rate, and arterial pH were decreased while myocardial blood flow did not change. Arterial lactate increased greatly, FFA did not change, glucose and O_2 decreased. Myocardial uptake and oxidation of FFA were greatly diminished and lactate uptake markedly elevated both periods following endotoxin. While under control conditions more than 70% of the energy utilization of the myocardium was derived from FFA, and about 30% from lactate, following the administration of endotoxin the contribution of FFA to myocardial energy utilization was greatly diminished and that of lactate was elevated. Thus a shift in utilization of substrates was observed favoring lactate and diminishing the contribution of FFA. These findings were similar to the changes in substrate utilization that were observed previously during experimentally produced hemorrhagic hypotension.

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ABSTRACT

Changes of myocardial substrate utilization have been studied in dogs under pentobarbital anesthesia following the administration of E. coli endotoxin. Myocardial substrate utilization under control conditions, at two hours, and again at three to four hours following endotoxin administration were compared. At both periods following endotoxin mean arterial blood pressure, heart rate, and arterial pH were decreased while myocardial blood flow did not change. Arterial lactate increased greatly, FFA did not change, glucose and O_2 decreased. Myocardial uptake and oxidation of FFA were greatly diminished and lactate uptake markedly elevated both periods following endotoxin. While under control conditions more than 70% of the energy utilization of the myocardium was derived from FFA, and about 30% from lactate, following the administration of endotoxin the contribution of FFA to myocardial energy utilization was greatly diminished and that of lactate was elevated. Thus a shift in utilization of substrates was observed favoring lactate and diminishing the contribution of FFA. These findings were similar to the changes in substrate utilization that were observed previously during experimentally produced hemorrhagic hypotension.

Endotoxin-produced shock has been widely used as an experimental model to investigate the hemodynamic, and more recently the metabolic alterations that occur in the course of the clinically important syndrome of shock. There is strong evidence that myocardial failure accompanies the final outcome of severe shock (2,4,8,10,11,17,19,20, 21). However, it is open to question whether myocardial functional derangements contribute to the deterioration of the condition, expecially in the early phases of the syndrome.

It has been demonstrated recently that marked changes in myocardial substrate utilization may be found quite early following the experimental production of hemorrhagic hypotension (23). There are indications that similar alterations of myocardial metabolism are also present in animals following endotoxin administration (18). Therefore it was of interest to investigate alterations of myocardial substrate utilization in the course of endotoxic shock and correlate them with the time course of the functional derangements of myocardial performance which has been recently reported by Hinshaw and collaborators (10,11). Thus, the present studies were undertaken to compare alterations in myocardial substrate utilization that occurred early following endotoxin administration with those that can be demonstrated several hours later.

MATERIALS AND METHODS

The experiments were performed on six adult mongrel dogs weighing 16.5 to 25.0 kg. The animals were anesthetized by the intravenous administration of sodium pentobarbital (30 mg/kg). Following tracheostomy the animals were respired with room air provided by a constant-volume respirator. Mean arterial blood pressure (MABP) was measured

through a catheter introduced into the aorta via one femoral artery. The other femoral artery was also cannulated for sampling of arterial blood. Thoracotomy was performed between the fourth and fifth ribs and the pericardium partially cut to facilitate placement of a catheter into the coronary sinus via the right jugular vein. A continuous slow saline drip was given in the coronary sinus catheter to keep it patent. No anticoagulant was administered to the animals.

A constant infusion of albumin-bound 1-^{14}C-palmitic acid (2 μCi/ml) and 9-10 ^{3}H-oleic acid (20 μCi/ml) was given to the animals at the rate of 0.2 ml/min. At least 60 minutes elapsed between the beginning of the labeled FFA infusion and the control blood samples.

Simultaneously drawn control blood samples were taken from the artery and coronary sinus for metabolite analyses followed by the intravenous administration of an LD_{90} of E. coli endotoxin (0.7 to 1.0 mg/kg, DIFCO, Detroit). Experimental blood samples were obtained 100-140' and 170-250' post-endotoxin.

Although the time interval between the two sets of experimental blood samples was 1-2 hours, the metabolic condition of the animals (with regard to the two major myocardial substrates) was quite stable. This can be judged by the unchanged arterial FFA concentration, by the similar arterial lactate concentration between the two experimental samples (Table 2) and also by the absence of a significant change in arterial FFA specific activity between the two samples (i.e. mean art. FFA SA of the first post-endotoxic sample = 2312 ± 821 dpm/μmole, and the change between the two arterial samples = 323 ± 233).

All blood samples were analyzed for the concentration of FFA (5), lactate (12), glucose (16), and O_2 and CO_2 concentrations (24). In

addition, the $^{14}CO_2$ content of each blood sample was also estimated (15) as were the ^{14}C and ^{3}H labeled FFA (6). In the arterial blood hematocrit and PH were also determined. Myocardial blood flow was measured by the I^{131}antipyrine method of Krasnow, et al (13).

The results are expressed as mean + SE for the control samples of all animals and as the mean change ± SE for all animals during each of the two time periods investigated following endotoxin administration.

All calculations were based on the assumption that labeled and unlabeled fatty acids are metabolized in the same way by the myocardium and that different individual fatty acids comprise a single pool with respect to uptake and release. The information presented in the tables was obtained by using the following equations (14).

The extraction of labeled FFA (E%), expressed as percent of the arterial level, was computed as follows:

$$E\% = \frac{\text{arterial radioactivity - venous radioactivity}}{\text{arterial radioactivity}} \times 100$$

Myocardial FFA uptake was calculated as follows:

FFA uptake (μmoles/min) =

$$\frac{\text{labeled FFA}_a - \text{labeled FFA}_v}{\text{labeled FFA}_a \text{ SA}} \times \text{plasma flow}$$

The rate of FFA oxidation to CO_2 was estimated by the following equation:

FFA oxidation (moles/min) =

$$\frac{^{14}CO_{2v} - {}^{14}CO_{2a}}{^{14}C \text{ SA FFA}_a} \times \frac{\text{plasma flow}}{1 - (\text{Hct}/100)}$$

FFA flux was determined by the method of Armstrong et al (1):

$$\text{FFA flux } (\mu\text{mole/min}) = \frac{\text{infused } ^{14}\text{C radioactivity (dpm/min)}}{^{14}\text{C SA FFA}_a}$$

Radioactivity is expressed in disintegrations per minute per milliliter. Subscripts a and v represent arterial or venous blood.

RESULTS

The hemodynamic charges following endotoxin administration are indicated in Table 1. It may be seen that mean arterial blood pressure decreased by about 50% at the earlier time period and fell even further later on. Heart rate decreased, but to a lesser degree than pressure. Myocardial blood flow decreased on the average, however the changes were not significant. Arterial blood PH decreased significantly at both time. Hematocrit remained unchanged.

Alterations in arterial metabolite concentrations and FFA flux following endotoxin administration are shown in Table 2. FFA concentration did not change throughout the experiments. Blood lactate was about equally increased at the earlier and at the later time of sampling. Arterial glucose decreased progressively in the course of the experiments. Arterial oxygen concentration showed a slight decrease in the later phase of the experiments. The decrease in FFA flux was not consistant enough to reach statistical significance.

Changes in myocardial FFA and O_2 metabolism are illustrated in Table 3. The fraction of arterial FFA that was removed through one passage is indicated under the heading of FFA extraction. Both of the labeled fatty acids were extracted by the myocardium to the same extent. Following endotoxin administration, extraction of both isotopes was

diminished. Although the extraction of ^{14}C is depressed more than that of ^{3}H, these differences are not significant. Both FFA uptake and oxidation diminished markedly following endotoxin administration. Myocardial RQ increased significantly during the first, and showed an average increase at the second sampling time which however was not statistically significant, although 5 of 6 animals exhibited an increase in this parameter. Myocardial oxygen uptake decreased significantly during both sampling periods.

Changes in arterial lactate concentration and myocardial lactate extraction and uptake are shown in Table 4. Arterial lactate concentration increased fro 1.3 μmoles/ml to close to 5 μmoles/ml. Thirty-eight percent of the arterial lactate was extracted under control conditions. This value decreased markedly following endotoxin administration. Myocardial lactate uptake increased considerably during both experimental periods.

It may be calculated that during the control phase of the experiments, 76% of the myocardial CO_2 production is accounted for by FFA oxidation and about 30% by lactate utilization (Table 5). These figures were markedly altered during endotoxin shock, indicating that FFA contributed to a lesser degree and lactate to a much greater extent to the myocardial energy metabolism than during the control period. These data and the method of calculation used in obtaining the values are shown in Table 5.

DISCUSSION

The possible role of the heart in the progression of irreversible shock is still debated. Hinshaw and collaborators (7,9) using a similar shock model to the one employed in these studies, although utilizing the

isolated working heart preparation, have not been able to demonstrate any functional alteration of the myocardium in the first 3-4 hours following the administration of endotoxin, but observed consistant signs of myocardial failure 4-6 hours following the insult. Therefore, it is of great interest to note that alterations in myocardial substrate utilization have been demonstrated several hours prior to signs of functional derangements (10,11) and that theses alterations persist for a number of hours.

The major changes in myocardial substrate utilization following endotoxin administration consisted of a depressed FFA oxidation and an increased utilization of lactate. These changes are quite similar to those observed following hemorrhagic hypotension (23). It is not clear at this time, what is the full physiological impact of this shift in myocardial substrate utilization. As pointed out previously (23) one might ascribe some survival value to the shift, or it may simply be a reflection of a general increase of lactate utilization by the whole body at a time when lactate influx and turnover greatly increase (i.e. in the early stages of shock). Such an occurrence would be in accord with as yet unpublished data obtained following severe hemorrhage in dogs.

The present studies indicated no evidence of myocardial hypoxia as judged by the absence of a diminished lactate utilization following endotoxin. A similar conclusion was also drawn in previous studies (18,23). It may also be noted that the myocardial E% of O_2 decreased at least during the experimental period, again indicating the absence of tissue hypoxia.

Although cardiac output was not estimated in the present studies,

it is likely that cardiac output was decreased along with the diminished MABP. Since the latter parameter decreased by almost 50% and by 30% at the two time periods of the study, and the myocardial O_2 consumption was decreased by only 22%, it seems that the efficiency of the myocardium was diminished following endotoxin administration. A similar conclusion was reached in previous studies involving endotoxin insult (18).

It is not evident from these studies whether the shift of substrate utilization is a direct response of the heart ot endotoxemia or it is related secondarily to the elevated arterial lactate concentration. The latter alternative appears to be the favored one at this time, sine similar metabolic alterations are also observed in other shock models (22,23) as well as after the administration of Na-lactate in control dogs (unpublished observations). At this time one can only speculate about the physiological significance of the preference shown by the myocardium to utilize lactate over FFA, which is similar to the preference exhibited by the myocardium of control dogs for ketone bodies over FFA (14).

The most significant finding of the present studies is the shift of myocardial substrate utilization following the onset of shock which precedes any functional deterioration of the myocardium by several hours. Whether this metabolic change contributes to the transition to subsequent irreversibility of the condition remains to be further investigated.

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TABLE 1.

CHANGES IN HEMODYNAMIC PARAMETERS, PH AND HEMATOCRIT

FOLLOWING ENDOTOXIN ADMINISTRATION (N=6)

		Change After	
	Control	100-140'	170-250'
Mean Arterial Blood Pressure (mm Hg)	122 ± 6	-57 ± 6	-35 ± 11
Heart Rate (Beats/min)	201 ± 10	-28 ± 11	-38 ± 12
Myocardial Blood Flow (ml/min·100 g)	106 ± 11	-15 ± 9	-16 ± 11
PH	7.43 ± 0.03	-0.19 ± 0.03	-0.16 ± 0.04
Hematocrit (%)	39.2 ± 2.2	+3.8 ± 2.6	+0.1 ± 1.4

Mean ± SEM

Table 2. CHANGES IN ARTERIAL METABOLITE CONCENTRATIONS AND FFA FLUX FOLLOWING ENDOTOXIN ADMINISTRATION

(N=6)

	Control	Change After 100-140'	Change After 170-250'
FFA (μmole/ml)	0.659 ±0.093	-0.001 ±0.107	+0.001 ±0.128
Lactate (μmole/ml)	1.312 ±0.239	+3.464 ±0.668	+3.666 ±0.853
Glucose (μmole/ml)	5.73 ±5.9	-1.51 ±0.56	-2.13 ±0.32
O_2 (μmole/ml)	6.67 ±0.61	-0.17 ±0.19	-0.36 ±0.18
Total Body FFA Flux (μmole/min)	683 ±216	-101 ±228	-185 ±108

Mean ± SEM

Table 3. CHANGES IN MYOCARDIAL FFA AND O_2 METABOLISM FOLLOWING ENDOTOXIN ADMINISTRATION

(N=6)

	Control	Change After 100-140'	Change After 170-250'
^{14}C-FFA Extraction (%)	53.0±4.6	-19.3±4.8	-20.2±2.7
^{3}H-FFA Extraction (%)	53.4±3.5	-11.8±5.2	-18.5±3.0
FFA Uptake (μmole/min. 100 g)	23.5±4.1	-11.0±3.4	-12.3±1.8
FFA Oxidation (μmole/min. 100 g)	19.3±2.5	-13.4±3.6	-7.7±4.6
MRQ	0.78±0.04	+0.16±0.06	+0.09±0.06
O_2 Extraction (%)	81.0±2.9	-10.0±2.5	-8.4±5.2
O_2 Uptake (μmole/min 100 g)	569±71	-137±34	-123±46

Mean ± SEM

Table 4. CHANGES IN MYOCARDIAL LACTATE METABOLISM FOLLOWING ENDOTOXIN ADMINISTRATION

(N=6)

	Control	Change After 100-140'	Change After 170-250'
Arterial Lactate (μmole/ml)	1.312 ±0.239	+3.436 ±0.668	+3.666 ±0.853
Lactate Extraction (%)	37.9 ±5.9	-14.4 ±4.8	-16.9 ±5.6
Lactate Uptake (μmole/min. 100 g)	48.5 ±10.5	+39.3 ±12.6	+38.0 ±11.5

Mean ± SEM

Table 5. CALCULATED CONTRIBUTION OF FFA AND LACTATE TO MYOCARDIAL CO_2 PRODUCTION FOLLOWING ENDOTOXIN ADMINISTRATION

(N=6)

		Change After	
	Control	100-140'	170-250'
Contribution of FFA (%)*	76±12	-53±13	-20±11
Contribution of Lactate (%)**	30±4	+35±12	+34±12

Mean ± SEM

*Calculated as [(FFA Oxid. x 17)/myocard. CO_2 prod.] x 100

**Calculated as [(Lact. Upt. x 3)/myocard. CO_2 prod.] x 100